

THE FATTY ACIDS OF BUTTER

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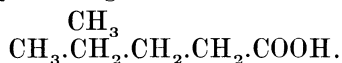
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I. THE NATURE OF THE CAPROIC ACID PRESENT IN BUTTER

In spite of the large number of analyses of butter fat carried out for technical purposes, there remains considerable uncertainty as to the constitution of the fatty acids it contains. Even the nature of the lower fatty acids present is not definitely established, for whereas the caproic acid isolated from butter fat is described in most of the standard works of reference¹ as iso-caproic acid on the alleged authority of Chevreul, Lewkowitsch² prefers to regard it as the normal acid from analogy with the other fatty acids in butter. No work on the constitution of the hexoic acid present in fats appears to have been carried out since its isolation by Chevreul³.

In Chevreul's pages no reference to the molecular structure of the acid is to be found, nor was the existence of two isomeric caproic acids established until fifty years after this work was published. The iso-acid was the first hexoic acid to be synthesised; Frankland and Kolbe⁴ saponified the cyan amyl obtained from the amyl alcohol of fermentation and obtained an acid possessing, therefore, the structure



It was not until twenty years later than the normal acid was discovered by Lieben and Rossi⁵, who carefully characterised the normal and iso-acids⁶, the solubility of the barium and calcium salts being especially characteristic. Up to this time, caproic acid had been found in butter³, in cocoanut oil⁷, in the flowers of *Satyrium hircinum*⁸, in the fusel oil of beet-molasses⁹, and in the fruits of *Ginkgo biloba*¹⁰. In none of these papers is any attempt made to identify this acid as either normal or iso, and, apparently, it is only because the iso-acid was synthesised twenty years before the normal that this error has crept into the literature. Franchimont and Zincke ascribe the normal structure to the hexoic acid obtained by oxidising the hexyl alcohol from *Heracleum*¹¹, and the caproic acid from the butyric fermentation of sugar was also identified as the normal acid by Lieben¹².

Examination of the data given by Chevreul indicates that the hexoic

acid of butter has probably the normal structure; he gives as the solubilities of the barium and calcium salts:—

100 parts of water at 10·5 dissolve 8·02 parts Ba salt.

„ „ „ „ „ 14 „ 2·04 „ Ca „

Lieben and Rossi⁶ give the following values:—

Normal Acid—

100 parts of solution at 18·5 contain 8·4967 grs. Ba salt.

„ „ „ „ „ „ „ 2·707 „ Ca „

Iso-acid—

100 „ „ „ „ „ „ 34·65 „ Ba „

„ „ „ „ „ „ 11·3 „ Ca „

Chevreul's data appear, therefore, to indicate that the acid which he obtained from butter possessed the normal structure.

The identification of the acid present in natural fats as normal or as iso-caproic acid is one of considerable importance; the existence of iso-caproic acid $(\text{CH}_3)_2\text{CH}\cdot\text{CH}_2\text{CH}_2\text{COH}$ would suggest its derivation from leucin by deamidisation and, hence, might indicate a close connection with protein metabolism. This view was put forward by Felix Ehrlich¹³, who suggested that the caproic acid present in neutral fats and that formed in fermentation, although hitherto regarded as the normal acid, might be mixtures of inactive di-methyl butyric with active methyl ethyl propionic acid, derived, respectively, from leucine and iso-leucine.

Ehrlich pointed out that fatty acids have been observed in the bacterial decomposition of proteins, leucine and caproic acid occurring together¹⁴; whilst the production of dextro-rotatory caproic acid from the decomposition of casein and elastin has also been observed by Neuberg¹⁵; in the former of these papers, however, no evidence is given as to the structure of the caproic acid present.

On the other hand, if the fatty acids which occur in butter and in cocoanut oil containing 4, 6, 8, 10, 12, 14, 16, 18 and 20 carbon atoms, respectively, consist of normal chains and are synthetic products, their formation from some simple starting product by an analagous series of reactions in which at each step two carbon atoms are added, appears probable.

It appeared desirable, therefore, to settle quite definitely the structure of the hexoic acid present in butter. The differentiation of the normal and iso series of acids is conveniently based on the examination

of the amides, those formed from the iso-acids melting at considerably higher temperatures than those which belong to the normal series.

This is shown in the following table:—

Normal	M. Point	Iso	M. Point
Butyric	115°	Dimethyl acetic	128°
Valeric	114-116	Iso-propyl acetic	127
Caproic	100	Iso-butyl acetic	118
Heptoic	96-97		
Caprylic	97-98	Ethyl-butyl acetic	102
Nonoic	99		
Caprin	108		
Undecanic	103		

The isolation of the caproic acid was carried out as follows:—

About 3,500 grs. of pure butter fat* having the constants Iodine value 38·7, Saponification value 232, Reichert-Meissl 29·9, were saponified with alcoholic potash and the fatty acids liberated. The mixture was then steam-distilled until the odour of the lower fatty acids was no longer markedly perceptible in the distillate. The distillates, neutralised with potash, had a distinct odour of the higher fatty alcohols; sufficient was not, however, obtained for further identification. After concentrating to a small bulk, the solution of the soaps was acidified and extracted with ether. 125 grams of acid thus obtained were distilled from a flask fitted with a Young's 8-pear fractionating column and separated into the following fractions:—

Boiling point	Weight	
Up to 160°	24 grs.	
163—168	43 „	Boiling point, butyric acid, 163 (7 mm.)
168—173	25 „	
173—178	10 „	Boiling point iso-valeric acid, 174
178—183	3 „	
183—188	2 „	Boiling point N. valeric acid, 186
188—193	2 „	
193—198	0·5 „	
198—203	2 „	Boiling point iso-caproic acid, 200
203—208	5 „	Boiling point N. caproic acid, 205
208—213	6 „	

The residue remaining in the flask (16 grs.) was then distilled without a fractionating column.

213—218°	1·2 grs.	
218—228	4·8 „	
228—238	2·7 „	
238—248	1·5 „	
248—258	1·7 „	
Residue	4·1 „	Solidified on cooling

Two grams of each of the fractions boiling at 173-178°, 183-188°, 198-203°, 203-208° and 208-213° were then converted by Aschan's method¹⁶ into the corresponding amides. From each of the fractions 198-203°, 203-208°

* Pure butter was obtained from Lovegrove's Dairy, Checkendon.

and 208-213° an amide separated, melting at 96-97°. This was recrystallised from dilute alcohol and, finally, from a mixture of chloroform and petroleum; the crystals melted at 99-100°.

Amides were then prepared from Kahlbaum's normal synthetic caproic and isobutyl acetic acids. After recrystallisation these melted at 99-100° and at 118°, respectively. The melting point of the former was not depressed when mixed with the amide of the caproic acid from butter. The fraction boiling from 198-213° contained, therefore, normal caproic acid, and gave no indication of the presence of the iso-acid.

The fraction boiling up to 160° was now examined for acetic acid. It consisted chiefly of ether, and was neutralised by the addition of 1.2 c.c. N.KOH. On acidifying the solution of the small amount of potassium salt, drops of an insoluble acid with the characteristic butyric odour at once separated, so that the quantity of acetic acid, if any were present, could only have been exceedingly small.

In the above experiment, the steam-distillation was not continued until the whole of the volatile acids had passed over, but was stopped when 4.5 per cent. of the total weight of acid had been received. The proportion of acids present in the distillate examined was approximately:—

Butyric acid	85 gr.	74.6 per cent.
Caproic acid	13 „	11.3 „
Caprylic and Capric acids	12 „		10.5 „	„
Solid acids	4 „	3.5 „

II. THE EXISTENCE OF UNSATURATED ACIDS IN BUTTER OTHER THAN OLEIC ACID

A careful fractionation of the methyl esters of the butter acids was undertaken in order to obtain evidence as to the possible existence of lower members of the oleic acid series. It is a remarkable fact that whereas the 18-carbon atom unsaturated acid is commonly identified in fats, the existence of lower members of the oleic series has only been shown in cod-liver oil¹⁷. The experiments now carried out appear to indicate the presence of a small quantity of a lower unsaturated acid accompanying the decoic acid present.

The butter used for the preparation gave the following values: Reichert-Meissl 26.84, Iodine 37, saponification 228.

1,900 grams of dried butter-fat were shaken up with 567 c.c. of a solution of sodium in methyl alcohol (4.536 N). The emulsion formed

was allowed to stand overnight and the esters next morning extracted with ether. After drying and evaporating off the ether on a water bath, 1,630 grams of liquid remained.

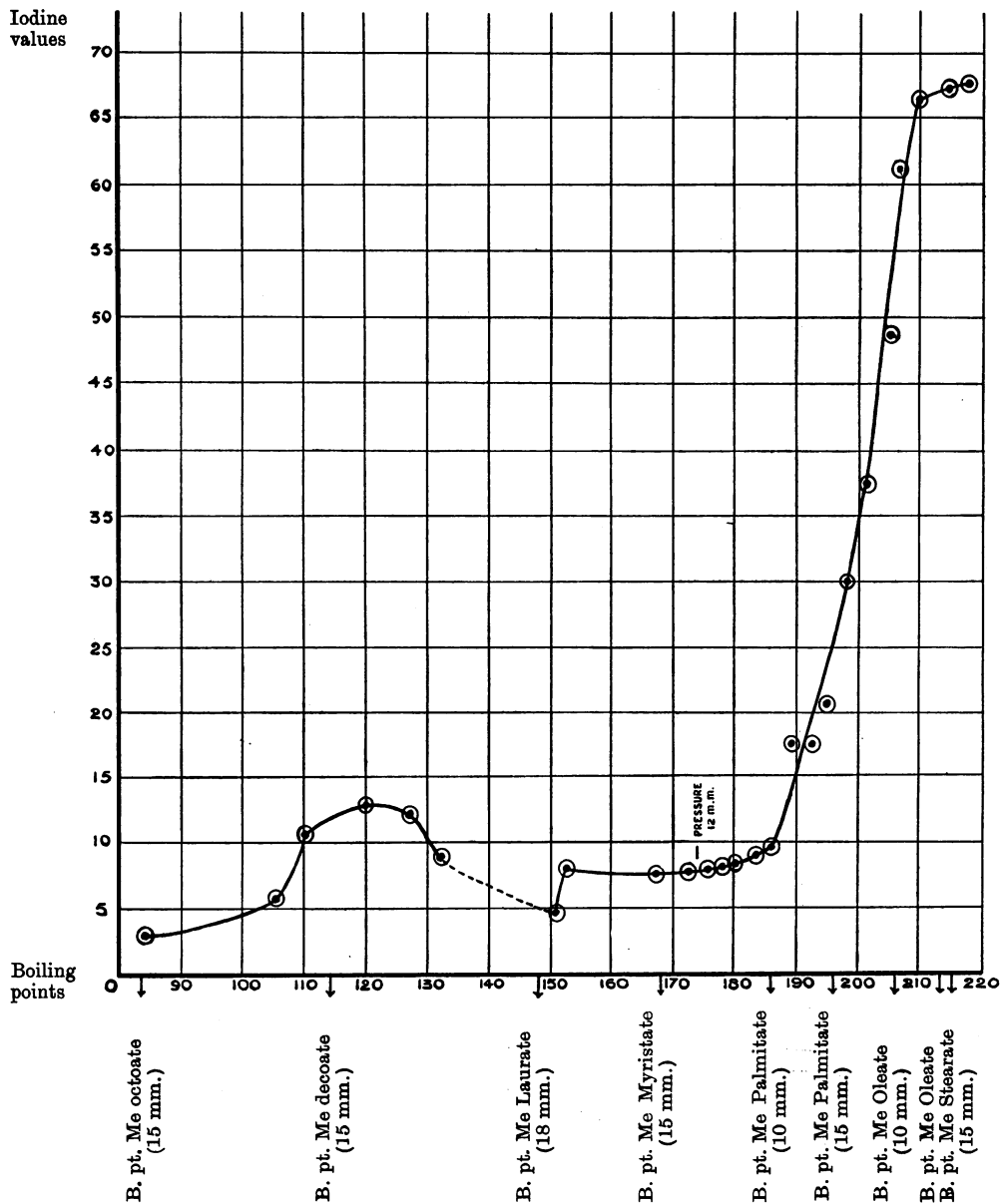
Examination for unsaturated acids.

The esters boiling above 160° under atmospheric pressure were then distilled under a pressure of from 10-15 mm., in a flask fitted with a Young's eight-pear fractionating column. The fractions were collected at first over from five to ten and then over every three degrees, and the iodine values of the different fractions determined; these are shown in the following table:—

Pressure	Boiling point	Weight of distillate	Iodine value	Saponification number	
15 mm. ...	80—85°	4 gms.	2.9	—	Boiling point methyl caprylate, 83° (15 mm.)
	85—90	6 "	—	—	
	90—95	—	—	—	
	95—105	7 "	5.4	—	
	105—110	8 "	10.4	—	Boiling point methyl caprate, 114° (15 mm.) Sapon. No. 302
	110—120	10 "	12.5	308.2	
	120—127	10 "	{ 12.0 11.9 }	307.3	
	127—132	8 "	8.7	282.9	
	132—147	—	—	—	Boiling point methyl laurate, 141° (15 mm.) Sapon. No. 262
	147—151	10 "	4.9	—	
10 mm. ...	151—152	{ 18 "	{ 7.8	—	Boiling point methyl myristate, 167.8° (15 mm.) Sapon. No. 232
	152—160	20 "	7.5	256.8	
	160—167	27 "	7.7	240.7	
	167—172	16 "	7.9	—	
	172—175	25 "	8.1	—	Boiling point methyl palmitate { 186° (10 mm.) Sapon. No. 208 { 196° (15 mm.)
	175—176	40 "	8.8	241	
	176—180	16 "	8.0	—	
	180—183	5 "	8.1	—	
12 mm. ...	183—186	14 "	8.8	—	Boiling point methyl oleate { 205.6° (10 mm.) Boiling point methyl stearate 212.3° (15 mm.) Sapon. No., methyl stearate, 188
	186—189	23 "	9.4	—	
	189—192	100 "	17.2	—	
	192—195	120 "	{ 17.5 17.2 }	221	
	195—198	89 "	20.5	—	Boiling point methyl oleate { 205.6° (10 mm.) Boiling point methyl stearate 212.3° (15 mm.) Sapon. No., methyl stearate, 188
	198—201	74 "	29.9	215	
	201—204	29 "	37.2	—	
	204—207	68 "	48.5	—	
	207—210	138 "	61.1	196	Boiling point methyl oleate { 205.6° (10 mm.) Boiling point methyl stearate 212.3° (15 mm.) Sapon. No., methyl stearate, 188
	210—215	215 "	66.6	—	
Residue in flask boiling above 220	215—218	114 "	67.3	—	
	218—220	26 "	67.8	—	
		32 "	—	—	
		1272			

DISTILLATION OF METHYL ESTERS OF BUTTER-FAT (obtained by Bull's method),
under 10-15 mm. pressure.

Curve showing relation of Iodine values to Boiling points.



The curve shows a marked rise in the iodine values of the fractions boiling from 105-127°. The marked maximum at 120° is followed by a gradual depression until 150° is reached, the iodine value remains constant whilst the boiling-point rises through 30° C., and then a rapid increase marks the distillation of the oleic acid. So far, attempts (based on the crystallisation of the lead, magnesium and barium salts) to isolate unsaturated esters from these lower boiling fractions have proved unsuccessful. Were oleic the only unsaturated acid present, a steady rise in the iodine value, with increase of boiling-point, might have been expected. The increase in iodine value in the fraction boiling from 110° to 130° appears to the author to furnish evidence of the presence of an unsaturated ester containing a less number of carbon atoms than oleic, possibly a decylenic acid since it passes over with the fraction containing methyl decoate (caprate). It is possible that traces of other unsaturated acids may also be present, and that the iodine values found for the lower fractions may not be due to traces of oleic acid.

Distillation of the ethyl esters.

A confirmatory experiment was carried out in which the fatty acids from the 3,500 grams of butter-fat used for the separation of caproic acid (cp. p. 453) were converted into the ethyl esters by boiling for several hours with an alcoholic solution of hydrochloric acid. After washing with a dilute solution of sodium carbonate to remove free fatty acids, and drying with CaCl_2 , 2,662 grams of esters were obtained, which were distilled under a pressure of 20-30 mm. and divided into three fractions:

- | | | | |
|---------------------------|---|---|--------------|
| (1) boiling up to 200° | - | - | 281 grams; |
| (2) boiling from 200-220° | - | - | 654 grams; |
| (3) boiling above 220° | - | - | 1,727 grams. |

The fraction boiling up to 200° was then distilled under a pressure of from 20-28 mm. in this case a six-pear fractionating column being used. The ethyl esters boil about 20° higher than the corresponding methyl esters. The iodine and saponification values of the different fractions were determined as in the previous experiment; a distinct increase in the iodine value was found in the fraction, the saponification value of which agreed with that required for the decoic ester.

Distillation of ethyl esters of butter fatty acids boiling up to 200° (20-30 mm.); (the more volatile acids having been partially separated).

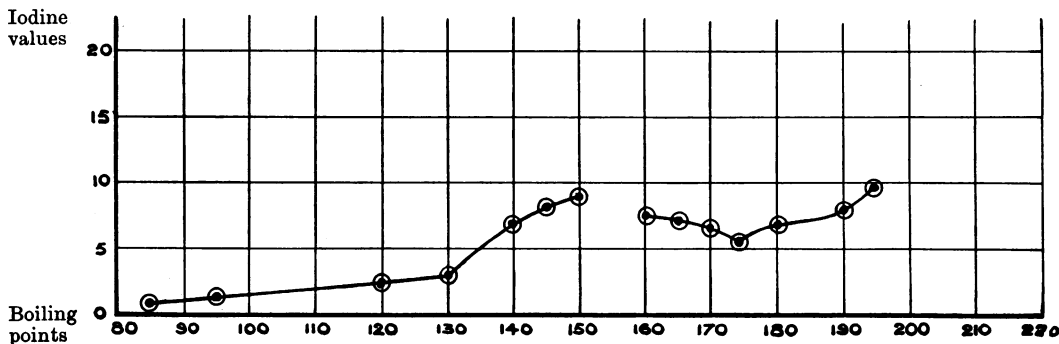
Pressure		Boiling point	Weight of Fraction	Saponification Number	Iodine Number	
25-28 mm.	...	80—85°	12.8	389.8	0.7	Sapon. No. ethyl hexoate = 388 (caproate)
		85—90	2.3	—	—	
		90—95	2.0	—	1.5	
		95—100	0.2	—	—	
		100—105	1.2	—	—	
		105—110	0.2	—	—	Sapon. No. ethyl octoate = 326 (caprylate)
		110—115				
		115—120	4.0	349.6	2.5	
		120—125	20.0	316.5	3.0	
		125—130				
		130—135	5	—	—	
		135—140	5.5	—	6.8	
		140—145	15.5	288.8	7.8	
		145—150	21	282.1	8.7	Sapon. No. ethyl decolate = 280 (caprate)
* 20 mm.	...	150—155	—	—	—	
		155—160	18	259.4	7.2	
		160—165	9	—	6.8	
		165—170	7	252.8	—	
		170—175	6	—	5.5	Sapon. No. ethyl laurate = 245
		175—180	18	238.9	6.5	
		180—185	3	—	—	
		185—190	8	—	7.4	
		190—195	14	222.6	9.6	Sapon. No. ethyl myristate = 219
		The distillation was not further continued.				

The distillation was not further continued.

* Distillation stopped here : continued on the following morning under a pressure of 20 mm.

DISTILLATION OF ETHYL ESTERS OF BUTTER-FAT (obtained by esterification of fatty acid with ethyl alcohol and HCl) under 20-25 mm. pressure.

Curve showing relation of Iodine values to Boiling points.



Three other experiments were carried out, in each of which between 300 and 400 grams of methyl esters were prepared and distilled. In every case the iodine values of the fractions corresponding to the decoic acid were higher than those of the succeeding fractions. The increase is not very large, but was present in five different specimens of butter examined, and it may be inferred therefore that it is generally present, and may be regarded as significant. It appears probable that the presence of a decylenic acid is indicated.

III. ON THE PRESENCE OF STEARIC ACID IN BUTTER

In view of the statements of Lewkowitsch and others (cp. Lewkowitsch, loc. cit.) that, when examined by the ordinary methods used in the examination of butter, only traces of stearic can be found, the higher boiling fractions of the methyl esters were examined for stearic acid. From the fractions in two experiments boiling at 210-215° a solid ester was separated, which, when twice re-crystallised from alcohol, melted at 37-38°, and on saponification gave an acid crystallised from alcohol in plates melting at 69°, and which was therefore identified as stearic acid. The quantity of stearic present was approximately estimated as about 10-15 per cent. of the total butter acids. Lewkowitsch has drawn attention to anomalies observed in the estimation of stearic acid when lower acids than palmitic are present (cp. Lewkowitsch, loc. cit., Vol. I, p. 453). The presence of considerable quantities of stearic acid in butter-fat was also found by Caldwell and Hurtle¹⁸, who state that the oleic acid of butter occurs chiefly in combination as an oleo stearo palmitin.

Reaction with sodium-nitro-prusside.

The distillate, boiling up to 140° under atmospheric pressure, gave a very low iodine value of between two and three units. The fraction boiling from 100-120° was heated with a solution of caustic potash, acidified with acetic acid and a solution of sodium-nitro-prusside and strong ammonia then added; a deep violet colour developed. The same fraction gave negative results when treated with Fehling's solution and with ammoniacal silver nitrate. The sodium-nitro-prusside reaction was obtained in two out of five experiments in which the esters had been prepared by Bull's method; but when the esters were prepared by an acid method of alcoholysis, in no case was the reaction obtained. This

reaction is especially characteristic of aceto-acetic acid and of acetone, and it is possible that these were present in those samples examined which gave positive results. In view of the inconstancy of the results obtained, it is possible that these products had been formed as the result of bacterial action. Further evidence as to the nature and origin of the substances producing this reaction is desirable, as the certain detection of aceto-acetic acid in butter would be a point of considerable importance.

CONCLUSIONS

1. No evidence of the presence of acetic acid was found in the butter examined.

2. The hexoic (caproic) acid present in butter possesses the *normal* structure; no indication of the presence of the iso-hexoic acid was obtained. It seems probable that of all the naturally existing specimens of caproic acid which have been described, only that occurring in the bacterial decomposition of proteins has a branched structure.

3. The proportion of stearic acid was estimated as from 10-15 per cent.

4. Evidence was obtained of the existence of lower members of the oleic acid series; the iodine value of the decoic ester fraction is appreciably greater than those of the fractions immediately preceding or following it. This may be regarded as indicating the presence of a lower unsaturated acid, possibly of a decylenic acid.

5. The sodium-nitro-prusside reaction, characteristic of aceto-acetic acid and of acetone, was given by the butyric ester fraction obtained from two out of five samples of butter, esterified by sodium methylate at ordinary temperature: the remaining three samples gave negative results. The reaction may possibly have owed its origin to the products of bacterial action; it was observed in two cases where Haller's acid method of esterification had been used.

REFERENCES

1. Abderhalden, *Biochemisches Hand-Lexikon*, Vol. I, p. 989; cf. Beilstein, *Handbuch der Organischen Chemie*, Vol. I, p. 432.
2. Lewkowitsch, *Chemical Technology and Analysis of Oils, Fat and Waxes*, 4th Edition, Vol. I, p. 122, 1909.
3. Chevreul, *Recherches sur les corps gras*, p. 134, 1823.
4. Frankland and Kolbe, *Annalen*, LXV, p. 288, 1848.
5. Lieben and Rossi, *Ann.*, CLIX, p. 75, 1871.
6. Lieben and Rossi, *Ann.*, CLXV, p. 113, 1873.
7. Fehling, *Ann.*, LIII, p. 406.
8. Chautard, *Jahresber.*, p. 340, 1864.
9. Müller, *Jahresber.*, p. 499, 1852.
10. Bechamp, *Ann.*, CXXX, p. 364.
11. Franchimont and Zincke, *Ann.*, CLXIII, p. 193.
12. Lieben, *Ann.*, CLXX, p. 89, 1873.
13. Felix Ehrlich, *Ber.*, XL, p. 2547, 1907.
14. Emmerling, *Ber.*, XXX, p. 1863, 1897.
15. Neuberg, *Biochem. Ztschr.*, I, p. 368, 1906.
16. Aschan, *Ber.*, XXXI, p. 2348, 1898.
17. Bull, *Ber.*, XXXIX, p. 3570, 1906.
18. Caldwell and Hurtley, *Trans.*, XCV, p. 858, 1909.